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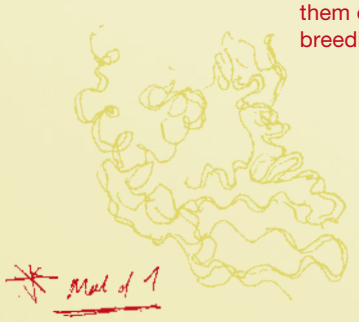
Enjoying apples **without regrets**

Deep-frozen samples of newly bred apples such as these form the basis of the analyses in Wilfried Schwab's laboratory.

In Germany, the average person eats almost 20 kilos of apples every year – and they're expected to be as crunchy, rosy-red and sweet as possible. More and more individuals, however, are allergic to fruit. In response to this situation, a team of researchers headed by Wilfried Schwab, Professor of Biotechnology of Natural Products at TUM, are working on a project to breed "allergy-friendly" apple varieties. If all goes well, consumers could find these varieties in stores by 2025.

Picture credits: Magdalena Jooss, Arne Dierend; Graphics: ediundsepp

The samples come from Osnabrück. Prof. Werner Dierend, right, from Osnabrück University of Applied Sciences, shows how he selects the best apple trees and registers them on the computer for further breeding.



No, there won't be apples to sample today. Emilia Romer briefly lifts up pieces of apple, chopped up into small bits and vacuum-packed in plastic film. "These are our investigation objects," she says, before immediately placing the sample back in the deep freezer. The designation P 98 10.10.2018 was barely visible on the packaging. "What you saw was an apple sample from tree 98, harvested on October 10, 2018," explains Romer. Researchers examining the biotechnology of natural substances at TUM on its Weihenstephan campus are searching for apple varieties that allergy sufferers will also be able to enjoy. Sample P 98 is one of hundreds that doctoral students Emilia Romer and Soraya Chebib are analyzing to determine the precise concentrations of a specific group of proteins known as Mal d 1 proteins. New varieties of apples suitable for allergy sufferers need to contain as little of these proteins as possible, as they are the main cause of apple allergies in Central and Northern Europe.

Almost five percent of German adults suffer from such food allergies. Even the very first bite into a piece of fruit or vegetable entails the risk of itching and rapid swelling of the lips, tongue and mucous membrane of the mouth. Thankfully, highly dangerous reactions culminating in anaphylactic shock are rare.

Completely allergen-free is not feasible

So, will the first allergen-free apples soon be on the markets? Wait a minute, not quite. Project leader Prof. Wilfried Schwab promptly warns against overly grandiose pronouncements: "We can't promise allergen-free produce. There will, however, be 'allergy-friendly' apple varieties."

The research has not reached that stage yet, though, and the scientists are keeping their cards close to their chest. What they will disclose, however, is that such apples could be available in selected stores by 2025. This work is enabled by a joint project financed by the Federal Ministry of Food and Agriculture in which TUM experts are contributing the analytics. The samples are exclusively delivered by post – from Osnabrück University of Applied Sciences (HS OS) in northern Germany.

Prof. Werner Dierend, holder of the Chair for Fruit Growing and Processing, remembers that, back in 2002, fruit farmers from the Züchtungsinitiative Niederelbe association (ZIN) asked whether he could help them with new apple varieties. ZIN is an association comprising 170 apple producers and wholesalers. A number of its members had identified a need to refresh and rejuvenate the market.

Image-boosting new varieties

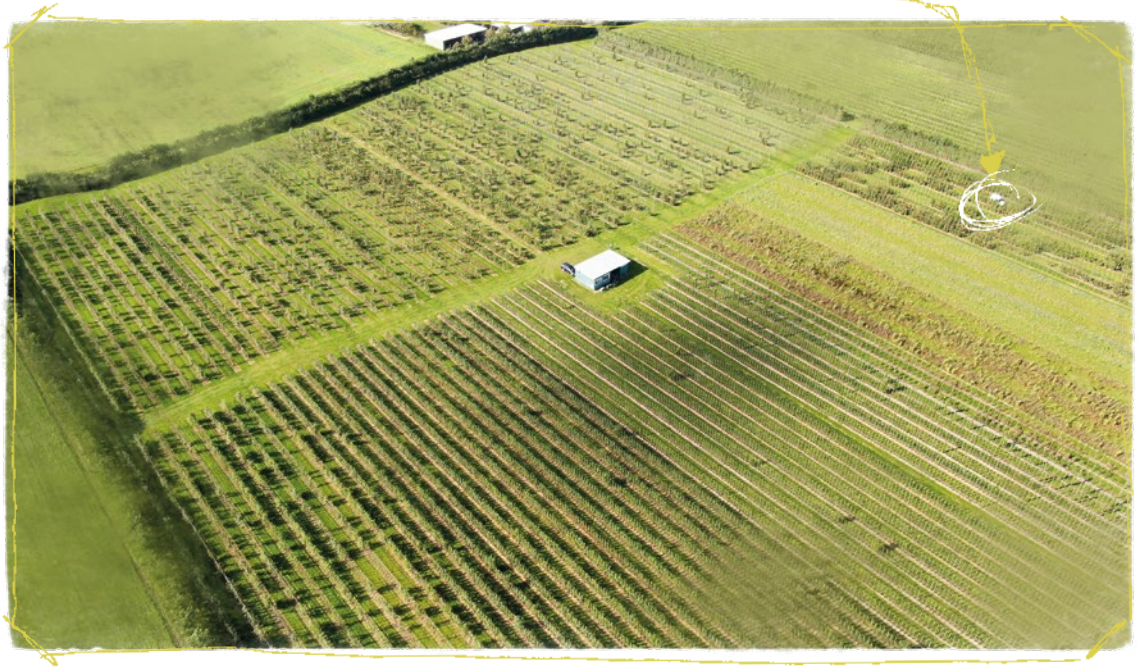
"Customers want crunchy, juicy apples," as Dierend emphasizes. What's more, he tells us, the most common apple varieties in Germany, Jonagold and Elstar, are no longer exactly the crunchiest. Generally speaking, the apple specialist explains, new apple breeds are announced every 15 to 20 years – for marketing and image reasons, but also because years of cultivation of varieties tends to lower the quality levels. With this in mind, in 2003, Dierend launched a breeding program together with the ZIN association. The objectives? Fresh, crunchy apple varieties suitable for cultivation in northern Germany, high yields and as pathogen-resistant as possible. And all in one apple variety, if you please.

The technique to achieve this is tried and tested. By way of controlled pollination the researchers from Osnabrück University of Applied Sciences and ZIN cross selected varieties whose descendants are to combine the desired characteristics of both parents. The university's contacts from all corners of the world helped to source suitable parent varieties. Selecting the best apple trees is crucial for the subsequent F1 generation. Year after year, the apple breeders evaluate flavor, resistance and yields. In total, the program is working on around 300 crossing batches. The new varieties stand in long rows in trial fields at ZIN and the HS OS. In 2016, the breeders were able to submit an application for protection for the first new apple variety to the Federal Plant Variety Office (BSA).

Initially, the topic of allergies had not been on their radar screen at all. "It was only in 2012, when we first learned that people at TUM can measure significant apple allergens, that we included this aspect in our program straight away," explains Dierend. ▷

"When we learned that researchers at TUM are able to measure significant apple allergens, we included that in our program straight away."

Werner Dierend



The Züchtungsinitiative Niederelbe's selection field is used to grow low-allergen apples. The best of an initial stock of trees (at bottom of image) are selected, while the others are cleared out. In the end, this leaves the mother trees for the new varieties (top center of image).

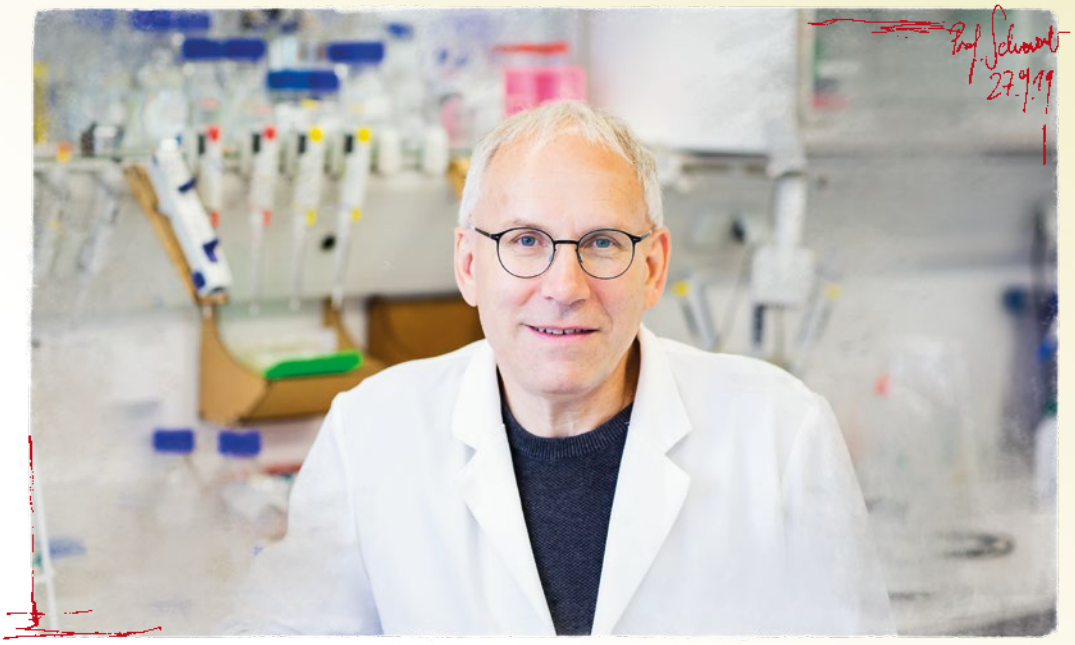
Picture credits: Magdalena Jooss, Arne Dierend; Graphics: ediundsepp

From white strawberries to apples

Wilfried Schwab also first became involved in allergen research by a lateral route, when he had initially started tweaking aromatic substances and pigments in fruits at TUM in 2003. It was the work of biochemist Prof. Cecilia Emanuelsson at Lund University in Sweden that first prompted him to track down allergens. Starting in 2004, Emanuelsson's research group discovered that some varieties of white strawberry contain very small amounts of a protein called Fra a 1 (the name of which is derived from the scientific name for garden strawberries, Fragaria ananassa). They also noted that patients with strawberry allergies were often better able to tolerate white varieties – not because of the lack of pigment but due to the low levels of Fra a 1. Today, this protein is seen as the most significant allergen in strawberries. At the same time, it is a member of a family of allergens with relatives in many other plants including tomatoes, plums and cherries. Its equivalent in birch pollen is called Bet v 1 (for Betula verrucosa, commonly known as weeping birch). The protein in apples is Mal d 1 (for Malus domestica, domestic apple trees).

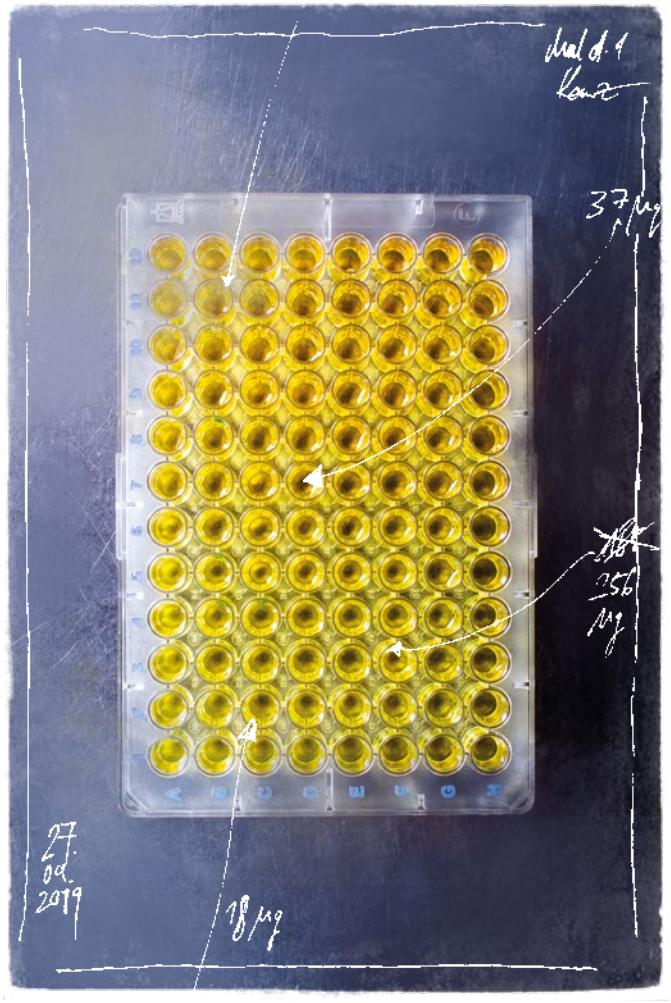
The function of proteins in plants is just as unclear as the question of why some people's immune systems react to the members of this family of proteins in particular. The high degree of similarity between these proteins explains the frequent cross-reactions in food allergy sufferers. According to the current hypothesis, if a person in Central and Northern Europe has a corresponding disposition, there is a high risk they will develop a sensitization to Bet v 1 in birch pollen. From there, it is just a short step to cross-reactions with food allergies. As a result, 50 to 75 percent of the individuals suffering from birch pollen allergies in Central and Northern Europe are also allergic to apples and other fruits.

Schwab's research group has had an antibody against allergens such as Fra a 1 at hand since 2012, which it used to develop measurement methods for the entire protein group – known as ELISA (enzyme-linked immunosorbent assay). Several years ago, his team was thereby able to demonstrate that strawberries and tomato varieties differed tremendously in terms of their allergen content. This opened the door to the notion of using plant breeding to search for varieties with the lowest level of allergens possible. The project to develop "allergy-friendly" apples is one of the first to attempt to implement the concept. Since 2016, ZIN, HS OS and TUM have collaborated on the project to breed low-allergen apples, drawing on funds provided by the Federal Ministry of Food and Agriculture. Ever since, sample after sample has arrived at Romer and Chebib's lab.



The analytical work is conducted in Wilfried Schwab's laboratory in Freising. The tests examine the level of allergens the apple samples still contain.

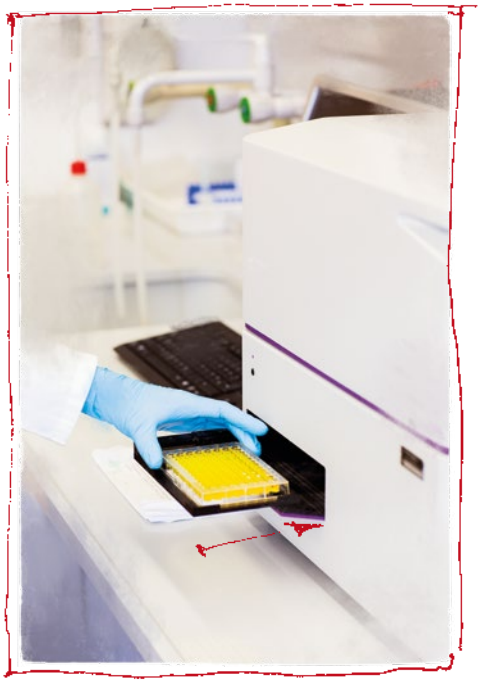
Protein Mal d 1 is then inserted in the 96 wells in microtiter plates. The researchers then pipette extracts from the apple samples and an antibody that binds to Mal d 1.



Predatory competition on the titer plate

The two researchers use standard microtiter plates for their analyses. To begin with, they always insert the same, precisely defined quantity of high-purity Mal d 1 proteins in each of the 96 wells in the microtiter plate. The researchers then pipette extracts from the apple samples and an antibody that binds to Mal d 1. The antibody binds with both the Mal d 1 proteins on the plate and the proteins from the apple. This continues until a balance is reached. ▷

The machine reads out the color values in the individual wells and thereby calculates the quantity of allergen Mal d 1 (protein structure shown at right).



Graphics: ediundsepp (based on a model in: C Seutter von Loetzen et al. (2012) NGL viewer. www.rcsb.org/structure/2LPX)
Picture credits: Magdalena Jooss

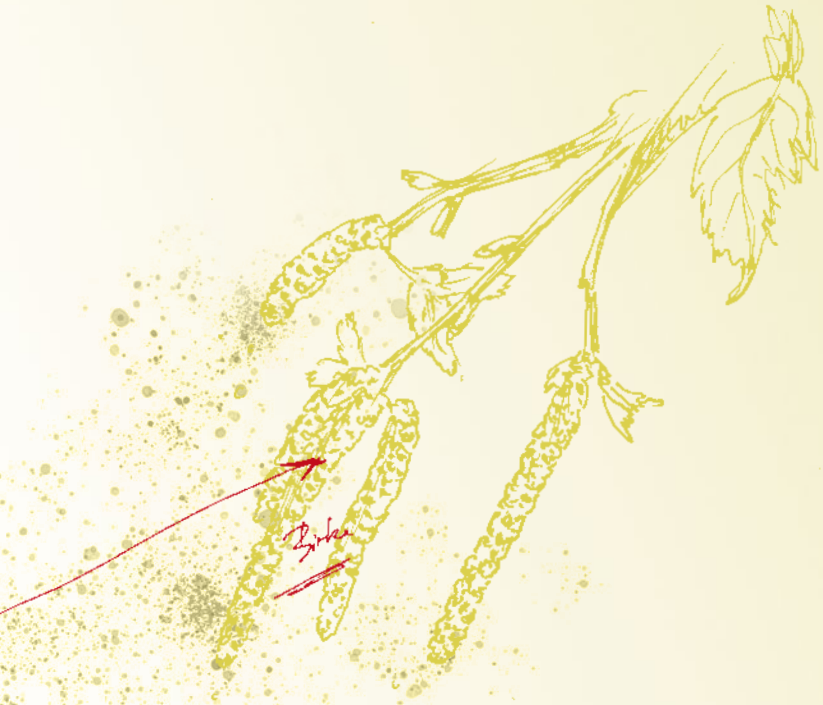
Prof. Wilfried Schwab

Wilfried Schwab is Professor of Biotechnology of Natural Products at TUM in Weihenstephan. Born in Franconia in 1961, he studied food chemistry at the University of Würzburg and was awarded his doctorate in 1989. Following a year at the Institute of Biological Chemistry at Washington State University in Pullman, USA, Schwab spent three years working for Hoechst AG and AgrEvo in Frankfurt. He returned to the University of Würzburg in 1994 and qualified as a professor in 1999. In 2003, Wilfried Schwab accepted an endowed professorship at TUM, and some five years later, the university made his professorship permanent. While Schwab does not rule out resorting to genetic engineering in plant breeding, he advocates first using and selecting the aspects available in the current diversity of fruit and vegetable varieties. Wilfried Schwab is married and has two children.

Once new varieties have been admitted to the national list, allergy-friendly applies could be available in selected stores as from 2025.

“We can’t promise allergen-free produce. But there will be ‘allergy-friendly’ apple varieties.”

Wilfried Schwab



The decisive factor: The more Mal d 1 an apple sample contains, the more antibodies attach to these molecules. In the end, a color reaction quantifies the number of remaining antibodies. In this way, the researchers are able to calculate how much Mal d 1 an apple contains. All this involves painstaking manual input – pipetting and washing, buffering and coloring. “Each titer plate takes us several hours to process and allows us to analyze twelve samples,” reports Chebib. The last step is quite fast, with a machine reading the color values of all 96 wells on the plate and the computer converting everything and reporting: The sample from tree P 98, collected on 10.10.2018, contains … well, so what exactly?

No, the researchers are not ready to disclose any precise findings just yet. They do offer an overall insight, however, namely that the variability in the new varieties is enormous. The values recorded can differ by a factor of 400 – to put this in context, this means that while some might contain 40 micrograms of Mal d 1 per gram of fresh apple, others may contain as little as 0.1 micrograms.

Tests on subject groups

It doesn’t take a genius to predict the people who might be suitable candidates to test some of the latter apples. Wilfried Schwab, however, continues to warn against oversimplification. He asserts that we know the Mal d 1 content does not allow us to draw definitive conclusions on the allergic reaction risks in each individual case. This is because there are dozens of protein variants related to Mal d 1, which can often appear in different quantities in different varieties of apple. It is also apparent that patients can experience very individual reactions to only specific variants. The antibody test in the TUM lab generally records all variants as a total value – but does not say anything about the proportions in which the different variants appear.

The final stage of the project is therefore testing on subject groups; since 2018, these subjects have regularly attended the Allergy Center at Berlin’s Charité hospital (Prof. Karl-Christian Bergmann) to eat apples from the breeding project with a low Mal d 1 content. The subjects are asked to eat 30 grams of apple and note down whether they experience allergic reactions and, if so, how severely. If the first sample is tolerated, they retry with 100 grams and record the results once again. This data will show which new apple varieties are the most “allergy-friendly” for as many allergy sufferers as possible.

That is to say, in Northern and Central Europe. That’s because a separate research project would have to be carried out for Southern Europe. The whole protein group of Mal d 1 & co. is not of any major significance for food allergies in that region. The assumed reason for this? Birch trees are fairly rare in Spain and Italy, so there is no incidence of initial sensitivity to birch pollen and subsequent cross-reaction to Mal d 1 & co. While there are also many people with food allergies in Southern Europe, they are responding to different proteins. That, however, is a story for another day.

■ *Bernhard Epping*

Picture credits: Magdalena Jooss, Arne Dierend; Graphics: ediundsepp